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The Influence of Cryopreservation on Changes in Diameter and Compliance of Allografts in an Animal Experimental Model

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Objective. *The purpose of this study was to analyze the influence of cryopreservation on changes in diameter and compliance of allografts.*
Methods. *Sixty aortic allografts implanted* in situ *in rats were analyzed. The animals were divided into four groups that received fresh or cryopreserved isogenic (Lewis to Lewis) grafts, or fresh or cryopreserved allogenic (Lewis to DA) grafts, respectively. The diameter and compliance of the grafts were then visually evaluated with the digital video camera recorder after 15, 30, 60, 90 and 120 days.*
Results. *Gradual increase in diameter and decrease in compliance in case of all allogenic and cryopreserved isogenic grafts were observed. The observed changes in cryopreserved grafts were smaller when compared with fresh grafts, however, the differences did not reach statistical significance.*
Conclusion. *Cryo preservation does not protect allografts from stiffening and dilatation.*

Keywords: Arterial allograft; Cryopreservation; Compliance; Animal model.

Introduction

Implantation of vascular allografts is one of the principal methods of vascular reconstruction following removal of infected grafts.[1,2] Unfortunately, although early results are very satisfying, there is a substantial number of long-term complications. The most important complications are segmental stenosis or dilatation, thrombosis, cracking of the wall or disruption of the anastomosis leading to development of pseudoaneurysms.[3,4] It can be assumed that development of these long-term complications results from remodeling of histological structure of blood vessels and changes in mechanical properties of the grafts. Both these phenomena are associated with chronic rejection reaction.[5,6] Taking into the account limited possibilities of antigen matching between recipient and donor of allograft, physical methods of preservation were proposed to decrease unfavorable consequences of chronic rejection reaction.[7] One of these methods is cryopreservation. The studies on the influence of cryopreservation on the mechanical properties of grafts have shown inconsistent results. There have been reports of beneficial influence of cryopreservation on the maintenance of cell function and mechanical properties.[8,9] On the other hand, the harmful effect of freezing on different cell populations, contractile function of the vessel wall and possibility of cracking of the vessels have been reported.[10,11] The purpose of this study was to analyze the influence of cryopreservation on changes in physical properties of allografts in an animal experimental model.

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Material and Methods

Donors

Sixty male Lewis rats were used as donors of the abdominal aorta.

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Recipients

Thirty male DA and 30 Lewis (Lew) rats were used. The recipients were divided into four groups of 15 rats each:

1. Group I—received fresh isogenic grafts (Lew to Lew)
2. Group II—received cryopreserved isogenic grafts (Lew to Lew)
3. Group III—received fresh allogenic grafts (Lew to DA)
4. Group IV—received cryopreserved allogenic grafts (Lew to DA)

All animals came from the Center of Experimental and Clinical Medicine of the State Academy of Science in Warsaw. The rats were housed and operated on in the animal laboratory of the Chair of Toxicology, University of Medical Sciences in Poznań. The animals were 14–16 weeks old and weighed from 250 to 350 g.

Anesthesia and operative technique

The rats were anesthetized with subcutaneous administration of ketamine (70 mg/kg of body weight) and xilazine (6 mg/kg of body weight). The peritoneal cavity was entered through a midline incision. The infrarenal abdominal aorta was exposed by blunt dissection. The lateral aortic branches were ligated with a monofilament suture 10/0 and a 20 mm segment of aorta was harvested from the donors. Directly before harvesting the external diameter (d_0) and the compliance (C_0) of the donor aorta were measured using the technique described below. In recipients, a 10 mm segment of the infrarenal aorta was excised and the harvested segment of aorta was implanted *in situ*. The anastomosis was performed in an end-to-end fashion with a continuous, nonabsorbable monofilament suture 9/0 (Ethilon, Ethicon). After completion of the anastomosis the aorta was declamped and quality of the anastomosis was assessed. Then the external diameter (d_1) and compliance (C_1) of the implanted segment of the aorta were measured. The peritoneal cavity was rinsed with a solution of povidone iodine and the abdomen was closed in a bilayered fashion with an absorbable suture 4/0 (Dexon). Immediately after the surgery all animals were given 10 ml of physiologic saline subcutaneously and placed in single cages.

Allografts preservation

The fresh allografts were harvested directly before implantation. After harvesting they were kept in a physiologic saline solution at a temperature of 4 °C for 20–30 min. The cryopreserved grafts were frozen in RPMI 1640 solution enriched with 10% DMSO, 10% FCS, 2 mmol/l glutamine and Na-pyruvate. The specimens were frozen at a constant, controlled rate of −1.1 °C/min using computerized freezer 'IceCube 1610', according to the experimentally determined gradient. When the temperature reached −80 °C, the frozen grafts were transferred to liquid nitrogen where they were kept for 10 days. Directly prior to implantation, they were thawed in a water bath at a temperature of 37 °C. The medium was gradually diluted with physiologic saline. The grafts were rinsed three times in physiologic saline and implanted as described above.

Measurement of the diameter and compliance of the grafts

After 15, 30, 60, 90 and 120 days three rats from every group were anesthetized. Two hundred units of unfractioned heparin were given subcutaneously and the graft was exposed with blunt dissection. To achieve better visualization of the graft, a piece of plastic was put posteriorly to the graft. After hemostasis was accomplished, the peritoneal cavity was filled with physiologic saline to prevent tissue desiccation. The external diameter and cyclic changes in the diameter of the grafts were recorded with a digital video camera recorder DCR-PC100E, 1 megapixel (Sony). The images were then transferred to a computer and processed with Pinnacle Studio 7.13 software. To avoid changes in hemodynamic parameters recording time did not exceed 10 s. The maximal diameter of graft corresponding to systolic peak and minimal diameter of the graft corresponding to an end diastole were measured through the slow motion option. The measurement of external diameter of the graft had the accuracy of 0.05 mm. Both absolute $[d_x - d_0]$ and relative $[(d_x - d_0)/d_0]$ changes in the external diameter of the grafts were calculated. The arterial blood pressure was invasively measured with the catheter introduced through the iliac artery. The compliance was calculated according to the following formula:

$$C = \Delta d/\Delta p (\mathrm{mm}^2/\mathrm{mmHg})$$

where C, compliance; Δd, systolic–diastolic difference in graft diameter; Δp, difference between systolic and diastolic blood pressure.

Histology and immunoenzymatic analysis

Segments of rat aorta obtained directly after harvesting and after thawing as well as specimens of arterial iso- and allografts obtained 15, 30, 60, 90 and 120 days after implantation underwent histology and immunoenzymatic examination. Paraffin sections 5 μm thick stained with hematoxilin and eosin and Masontrichrome were prepared for examination. Five micrometer thick sections were staining with antibodies against Actin (Immunotech), Vimentin (Immunotech), B and T lymphocytes (MRC OX3, Serotec, Ki-T1R), endothelial cells (OX43, Sera-Lab), macrophages (Ki-M2R) and proliferating cells (Ki-S3R). All antibodies of Ki-…R series came from Institute of Immunology of CAU-Kiel.[12]

The study was approved by the Ethics Commitee at the Karol Marcinkowski University of Medical Sciences in Poznan.

The data were analysed using the Statistica software package (StatSoft). The nonparametric Wilcoxon test was used to compare changes within the groups and nonparametric Mann–Whitney test was used to compare the differences between the groups. Differences were considered significant with $p<0.05$.

Results

External diameter of the graft

The mean diameter of the infrarenal aorta in donor Lewis rats was 1.56 mm ($\pm$0.2). The preservation of fresh grafts in physiologic saline at 4 °C for 20–30 min did not influence the diameter of the graft. After the first 15 days there were no changes in the diameter of the grafts in animals from group I. Starting from the 30th day gradual dilatation of the grafts was observed. A mean increase of 10.7% in the diameter of the grafts from the day of implantation to 120th day was noted ($p=0.013$). For cryopreserved isogenic grafts a 15.3% increase in the diameter of the grafts was observed directly after implantation ($p=0.042$). In further measurements a gradual increase in diameter of the grafts was observed. At the 120th day 37.2% dilatation was noted. In animals from group III slight but statistically insignificant decrease in diameter of the grafts directly after implantation was noted. Then the diameter of the grafts increased gradually, with the fastest growth between 30th and 60th day. The overall increase in diameter in group III was 62%. In group IV, a mean 22.1% dilatation of the grafts directly after implantation was observed ($p=0.006$). Subsequent measurements showed further dilatation of the grafts with the fastest growth between the 60th and 90th day. The overall increase in diameter in group IV was 54.8% ($p<0.001$). Taking into the account the dilatation related to cryopreservation, the relative increase in diameter in group IV was 26.8% ($p=0.002$). Both absolute and relative increases in diameter of the grafts were significantly higher in groups III and IV when compared to group I ($p<0.002$). There were no statistically significant differences between changes in groups II, III and IV ($p=0.93$) (Fig. 1).

Compliance

Compliance of the abdominal aorta in donor rats prior to harvesting was $12.3\pm1.2\ \mathrm{mm}^2/\mathrm{mmHg}$. In the beginning there was a decrease in compliance of the grafts in animals from group I. From the 60th day the compliance increased to reach 103.9% of the initial value on the 120th day ($p=0.004$). The compliance of cryopreserved isogenic grafts measured directly after implantation was increased by 5.1%. Starting from the 15th day the compliance in this group decreased to reach 92.3% of the initial value on the 120th day ($p=0.056$). In animals from group III the short-lasting increase observed on the 15th day ($p=0.038$) was followed by gradual decrease in the compliance. At the final measurement the compliance was decreased by 15.02% comparing to the initial value ($p<0.001$). In animals from group IV significant increase of compliance directly after implantation was observed (6.7%, $p<0.001$). This increase in compliance in this period was significantly higher when compared to other groups ($p<0.039$). Starting from the 30th day gradual reduction of compliance was noted. At the final measurement the compliance was decreased by 7.72% compared to the initial value ($p<0.001$) (Fig. 2).

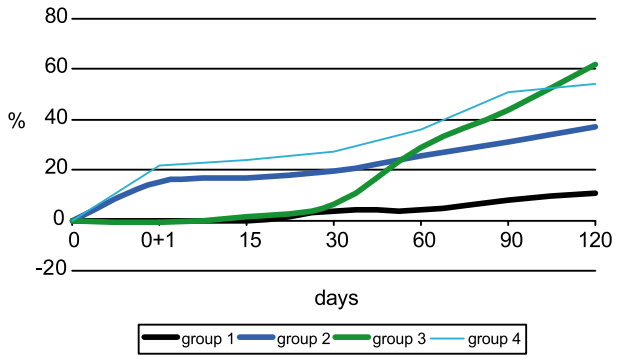

Fig. 1. The relative changes in the external diameter of aortic grafts within 120 days following implantation. Group I—fresh isogenic grafts, group II—cryopreserved isogenic grafts, group III—fresh allogenic grafts, group IV—cryopreserved allogenic grafts.

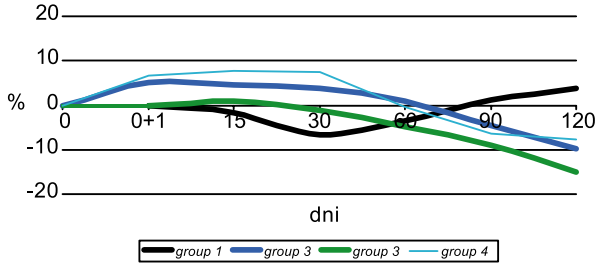

Fig. 2. The relative changes in the compliance of aortic grafts within 120 days following implantation. Group I—fresh isogenic grafts, group II—cryopreserved isogenic grafts, group III—fresh allogenic grafts, group IV—cryopreserved allogenic grafts.

Thus, only in animals from group I, we demonstrated a relative increase of compliance at the end of the observation period. In the remaining three groups the compliance decreased. There were significant differences in the relative changes of compliance between group I and groups III and IV ($p=0.002$). The differences between groups II, III and IV were not statistically significant ($p=0.21$).

Histology and immunoenzymatic analysis

Analysis of cryopreserved grafts after thawing

In all cryopreserved vessels total loss of the endothelium was observed. The spatial structure of the media was generally intact when compared with fresh specimens. There were small foci of unstained cells between layers of myocytes. A concentric arrangement of elastic fibers was maintained in all layers without any signs of fragmentation. Regardless of antigen matching of the specimens and method of preservation the defects in the intimal lining were filled with endothelium between 15th and 30th days of observation (Fig. 3).

Analysis of fresh isogenic grafts (group I)

No smooth muscle or inflammatory cells were demonstrated within the intima in any of the study periods. There were no significant changes in the structure of media and adventitia. The greatest intensity of T lymphocytes and macrophages infiltrates were present on the 30th day of the observation. These gradually resolved during the rest of the study period. Starting from the 15th day, proliferation of fibroblasts in the adventitia was noted. Later in the observation period adventitial fibrosis was demonstrated.

Analysis of frozen isogenic grafts (group II)

Early in the observation period intimal infiltrates of T lymphocyte and macrophages were noted. These

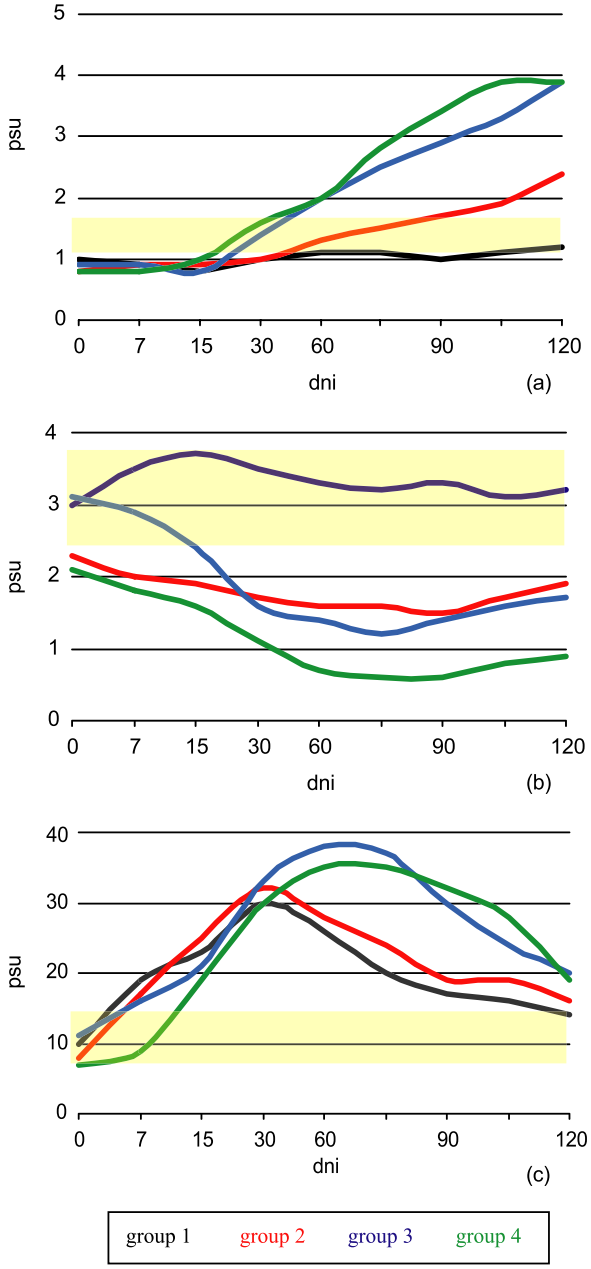

Fig. 3. Change in number of cell nuclei of all three layers of the vascular graft implanted in animal model in relation to duration of functioning. [a] intima; [b] media; [c] adventitia. The yellow area marks a normal range.

resolved completely after 60 days. Beginning from 30th day a few cells with positive reaction to Ki-S3R and MRC OX3 antibodies and to monoclonal antibody against actin and vimentin started to appear.

Starting from the 7th day significant decrease in number cell nuclei was observed in the media. Starting from the 15th day scarce infiltrates of T lymphocytes and macrophages occurred and from 30th day the proliferation of fibroblasts was increased.

Within the adventitia, intensive infiltrates of macrophages between 7th and 90th day and significant increase in amount of fibroblasts beginning from the 30th day were observed in contrast to group I.

C4. Fresh and cryopreserved allografts (groups III and IV)
The nature and dynamics of the changes occurring in structure of both fresh and cryopreserved allografts were comparable. In the intima, starting from the 15th day infiltrates of T and B lymphocytes and macrophages and were demonstrated in the subendothelial layer. Beginning from the 30th day cells with positive staining with Ki-S3R and, MRC OX3 antibodies and antibodies against actin and vimentin emerged. Progressive proliferation of smooth muscle cells and fibroblasts was accompanied by constant increase of thickness of intima. In the last study period cell density was significantly greater than seen initially ($p<0.002$). While there were no statistically significant differences in the changes of cell density between group III and IV ($p=0.554$), the changes in groups III and IV was significantly greater when compared to group I and II ($p<0.006$).

Within the media, from the 7th day the number of cell nuclei decreased. In the case of frozen grafts the initial number was 30% smaller than in fresh grafts and a similar difference was maintained throughout entire observation period. The number of cells reached the lowest level between 30th and 60th day of the study. In this period, only isolated muscle cells surrounded by inflammatory cells were seen. At the same time cells with a positive reaction to vimentin gradually increased. The broken integrity of elastic fibers, that was observed in six specimens, was the entry point for migration of cells from the media and adventitia to the intima.

Within the adventitia, rapid increase of cell number was secondary to an influx of T lymphocytes and macrophages. The intensity of infiltration reached its peak on the 60th day of the observation. On the 90th and 120th days of the study single mononuclear cells mainly macrophages and B lymphocytes were present. Between the 15th and 90th days the changing number of proliferating cells (positive reaction for Ki-S3R and mAb against vimentin) were observed. The changes in number of cells between extreme measurements was insignificantly greater in group IV ($p=0.554$). The changes in number of cells in both allogenic groups differed significantly from the range of changes in group I ($p=0.017$).

Discussion

The relation between long-term complications and remodeling of vascular grafts has been previously demonstrated. Both the intensity and duration of chronic rejection reaction are related to antigenicity of the transplanted material.[5,6] Cryopreservation is one of the methods to reduce the antigen load of the donor graft.[13] According to Giglia[8] and Petersen[14] the process of controlled freezing is supposed to limit the chronic rejection reaction and decrease 'immunologic' dilatation of allografts. They found that cryopreservation eliminates aneurysmal dilatation in an animal model. These results have not been confirmed in our study. Indeed we found that cryopreservation limited dilatation and compliance reduction, but the extent of these changes was nonetheless significantly bigger than in fresh isogenic grafts. Moreover, cryopreservation in the isogenic model increased diameter and decreased compliance when compared to fresh isogenic grafts. The differences between the results of Giglia and Petersen and ours could be due to difference in observation periods. Their study lasted only 8 weeks while ours was 10 weeks longer. We observed the acceleration of dilatation and changes in compliance 8 weeks after implantation, thus shorter observation periods would not be enough to detect these changes. The failure of cryopreservation to significantly limit inflammatory reaction and extent of histological remodeling following implantation of allografts has been reported by other authors.[15,16]

A Standardized process of cryopreservation enables the maintenance of intact spatial structure of collagen and elastin fibers.[17,18] However, cryopreservation and storage of the grafts are associated with significant loss of smooth muscle cells and changes in their function.[19] This results in dilatation of thawed grafts and transient growth of their compliance.[20]

Changes of elastic properties of vascular grafts are accompanied by remodeling of the vessel wall expressed by its thickening and domination of intimal hyperplasia.[20] These processes are stimulated by the inflammatory reaction within the graft wall induced by necrotic lesions occurring during the preservation process.[15]

Although normal morphology is apparently maintained, function of cryopreserved endothelial cells is modified. Isolated and cultured cell maintain their fibrinolytic properties and ability to excrete prostacyclins, however, this function is attenuated with long-lasting preservation.[21] The endothelium-dependent contractility of smooth muscle cells mediated by thrombin and acetylcholine is similarly impaired.[22]

This functional impairment is permanent and is not reversed even during long-term *in vitro* culture.

The loss of ability to withstand high pressure by endothelial cells seems extremely important,[21] and may be responsible for the total loss of endothelial cells by blood flow in the 1st day following implantation.[23] Thickening of the intima is the next critical finding. The first signs of this process can be observed in small arteries as soon as 7 days after transplantation and these initial changes are caused by infiltrations of inflammatory cells.[24] Starting from the 14th day gradual migration of smooth muscle cells from media to the intima was observed.[25] The process of cell migration is enhanced by increased activity of metalloproteinases, mainly 1, 3 and 9, which are responsible for degradation collagen and elastin elements.[14,26] Smooth muscle cells migrating to the intima undergo proliferation. Some authors report coexisting phenotype transformation of proliferating myocytes.[25,27] This transformation involves gradual loss of ability to contract and to produce α-actin, typical functions of mature cells, and replacement with a mechanism of synthesis and excretion of β-actin, which typical for fetal myocytes.[27] This type of transformation can be responsible for changes in the structure of matrix.[28,29] Campbell suspects, that phenotype transformation results from abolition of endothelial control after the endothelium is exfoliated. In cultures of myocytes the process of phenotype transformation is inhibited in the presence of endothelial cells but not fibroblasts.[27]

Gradually decreasing number of myocytes is the main change in media. Cryopreservation alone is responsible for death of half of the cells. Such a crucial difference in comparison with percentage of living endothelial cells is most frequently explained by the limited possibility of cryoprotectant penetration to deeper layers of the arterial wall.[13,21] Further destruction is done by inflammatory cells, which are mainly present in the external part of the intima. The severity of this process is related to the degree of antigen mismatch.[24] The third mechanism responsible for decreasing number of smooth muscle cells in the media of the allografts, namely apoptosis, is more and more often mentioned. Hirsch and Bergese believe that this process is responsible for the death of approximately 10% of myocytes and involves mainly areas of the vessel wall free of inflammatory infiltrate.[11] Although apoptosis could be generally observed within 2 weeks following implantation, the timing of the peak intensity of this process depends on the degree of antigen matching between a donor and a recipient. In the case of lack of compatibility the peak intensity occurs between the second and 14th day while in case of good matching between the 8th and 20th day.[11]

Both cryopreservation and inflammatory reaction accompanying graft implantation augment natural degradation of elastin and collagen fibers.[30] Bujan and Bellon showed that increased activity of metalloproteinases (MMP) 1, 2 and 9 can be observed in thawed and kept alive cryopreserved arterial allografts.[15,26] The activity of MMP-1, responsible for degradation of collagen, increases directly after thawing.[15] However, MMP-9, which is not present in fresh and cryopreserved vessels, is supposed to play a crucial role in tissue remodeling following transplantation.[26]

Since, the smooth muscle cells lose their ability to produce elastin the lost elastin fibers cannot be replaced.[31] Later on collagen, mainly type I and III, is synthesized by the myocytes and fibroblasts.[30] The synthesis of collagen is stimulated by the co-existing inflammatory process, that leads to increased amount of fibroblasts and increased expression of procollagen I and III genes.[32] Both donor fibroblasts, the ones that survived cryopreservation, and recipient fibroblasts migrating to the graft may be involved in that process. Analysis of histology specimens in rats showed that in the majority of cases remodeling slows gradually after approximately 90 days which correlates with the changes in compliance of the grafts.

In our *in vivo* study, a decrease in diameter of transplanted aortas from Lew recipients to DA donors was observed. The difference in arterial blood pressure between Lew and DA rats may be responsible for this fact. The lower blood pressure in DA rats causing lower stretching forces could result in both decrease of diameter and transient growth of compliance in group III.

Although the process of gradual dilatation of allografts may lead to development of late clinical complications, it may also be beneficial to some extent. It maintains internal diameter of the graft in the presence of intimal thickening that occurs in implanted vessels irrespectively of the degree of antigen matching and the method of preservation. It also enables stabilization of wall tension in the presence of its growing stiffness, which reduces the differences in mechanical properties between contiguous segments of the vessels.[33]

In conclusion, the implantation of cryopreserved isogenic and fresh and cryopreserved allogenic grafts is associated with their subsequent dilatation and decrease of their compliance. The cryopreservation does not significantly protect allografts from dilatation and decrease of compliance.

References

1 Gabriel M, Pukacki F, Chęciński P, Oszkinis G, Stanisic M, Zielinski M *et al*. Current options in prosthetic vascular graft infection: comparative analysis of 63 consecutive cases. *Langenbecks Arch Chir* 2004;**389**:272–277.

2 Kieffer E, Bahnini A, Koskas F, Ruotolo C, Le Blevec D, Plissonnier D. *In situ* allograft replacement of infected infrarenal aortic prosthetic grafts: results in forty-three patients. *J Vasc Surg* 1993;**17**:349–355.

3 Gabriel M, Pukacki F, Dzieciuchowicz Ł, Oszkinis G, Chęciński P. Cryopreserved arterial allografts in the treatment of prosthetic graft infections. *Eur J Vasc Endovasc Surg* 2004; **27**:590–596.

4 Vogt PR, Brunner-LaRocca H-P, Lachat M, Ruef C, Turina MI. Technical details with the use of cryopreserved arterial allografts for aortic infection: influence on early and midterm mortality. *J Vasc Surg* 2002;**35**:80–86.

5 Gabriel M, Fändrich F. Estimation of graft arteriosclerosis after allogeneic fresh and cryopreserved aortic transplantation in the rat. *Transplant Proc* 2002;**34**:711–712.

6 Häyry P, Isoniemi H, Yilmaz S, Mennander A, Lemström K, Räisänen A *et al*. Clinical and experimental chronic allograft rejection. *Transplant Proc* 1994;**26**:1702–1703.

7 Nataf P, Guettier C, Bourbon A, Nappi F, Lima L, Dorent R *et al*. Influence of arterial allograft preparation techniques on chronic vascular rejection: a histological study. *Transplant Proc* 1996;**28**:2890–2892.

8 Giglia JS, Ollerenshaw JD, Dawson PE, Black KS, Abbot WM. Cryopreservation prevents arterial allograft dilation. *Ann Vasc Surg* 2002;**16**:762–776.

9 Vogt PR, Brunner-La Rocca HP, Carrel T, von Segesser LK, Ruef C, Debatin J. Cryopreserved arterial allografts in the treatment of major vascular infection: a comparison with conventional surgical techniques. *J Thorac Cardiovasc Surg* 1998; **116**:965–972.

10 Chamley-Campbell JH, Campbell GR. What controls smooth muscle phenotype? *Atherosclerosis* 1981;**40**:347.

11 Hirsch GM, Kearsey J, Burt T, Karnovsky MJ, Lee T. Medial smooth muscle cell loss in arterial allografts occurs by cytolitic cell induced apoptosis. *Eur J Cardiothorac Surg* 1998;**14**:89–97.

12 Wacker HH. Immunohistochemische Erkennung der Zellen des lymphatischen Gewebes in der Ratte. In: Wacker HH, ed. *Sinuswandzellen. Immunakzessorische Zellen des Lymphknotensinus*. Germany: Gustav Fischer, 1994:29–42.

13 Sitzmann JV, Imbembo AL, Ricotta JJ, McManama GP, Hutchins GM. Dimethylsulfoxide-treated, cryopreserved venous allografts in the arterial and venous systems. *Surgery* 1984;**95**:154–159.

14 Petersen MJ, Abbot WM, H'Doubler PB, L'Italien GJ, Hoppel BE, Rosen BR *et al*. Hemodynamics and aneurysm development in vascular allografts. *J Vasc Surg* 1993;**18**:955–964.

15 Bellon JM, Gimeno MJ, Pascual G, Garcia-Honduvilla N, Dominguez B, Bujan J. Arterial damage induced by cryopreservation is irreversible following organ culture. *Eur J Vasc Endovasc Surg* 1999;**17**:136–143.

16 Neves JP, Gulbekian S, Ramos T, Martins AP, Caldas MC, Mascarenhas R *et al*. Mechanism underlying degeneration of cryopreserved vascular homografts. *J Thorac Cardiovasc Surg* 1997;**113**:1014–1021.

17 Warshaw DM, Mulvany MJ, Halpern W. Mechanical and morphological properties of arterial resistance vessels in young and old spontaneously hypertensive rats. *Circ Res* 1979;**45**:250–259.

18 Nataf P, Hadjiisky P, Lechat P, Peuchmaurd M, Guettier C, Mougenot N *et al*. Viability of cryopreserved arteria wall: enzyme-histochemical and physiological markers. *Cell Mol Biol* 1994;**40**:803–810.

19 Walker-Caprioglio HM, Trotter JA, Mercure J, Little SA, McGuffee LJ. Organization of rat mesenteric artery after removal of cells of extracellular matrix components. *Cell Tissue Res* 1991; **264**:63–77.

20 Gabriel M, ed. *Experimental and clinical studies on processes accompanying implantation of vascular grafts in sterile and infected environment*. Poznan: WNAM, 2004:66–86.

21 Brockbank KGM, Donovan TJ, Ruby ST, Carpenter JF, Hagen PO, Woodley MA. Functional analysis of cryopreserved veins. *J Vasc Surg* 1990;**11**:94–102.

22 Elmore JR, Gloviczki P, Brockbank KGM, Miller VM. Cryopreservation affects endothelial and smooth muscle function af canine autogenous saphenous vein grafts. *J Vasc Surg* 1991; **13**:584–592.

23 Faggioli GL, Gargiulo M, Giardino R, Pasquinelli G, Preda P, Stella A *et al*. Long term cryopreservation of autologous veins in rabbits. *Cardiovasc Surg* 1994;**2**:259–264.

24 Carpenter JP, Tomaszewski JE. Human saphenous vein allograft bypass grafts: immune response. *J Vasc Surg* 1998; **27**:492–499.

25 Geraghty JG, Stoltenberg RL, Sollinger HW, Hullett DA. Vascular smooth muscle cells and neointimal hyperplasia in chronic transplant rejection. *Transplantation* 1996;**62**:502–509.

26 Bujan J, Pascual G, Garcia-Honduvilla N, Gimeno MJ, Jurado F, Carrera-San Martin A. Rapid thawing increases the fragility of the cryopreserved arterial wall. *Eur J Vasc Endovasc Surg* 2000;**20**:13–20.

27 Campbell JH, Campbell GR. Cellular interactions in the artery wall. In: Huynor S, Ludbrook J, Shaw J, McGrath M, eds. *The peripheral circulation*. New York: Elsevier, 1984:33.

28 Dilley RJ, McGeachie JK, Prendergast RJ. A review of the proliferative behaviour, morphology and phenotyps of vascular smooth muscle cells. *Arteriosclerosis* 1987;**63**:99–103.

29 Nataf P, Guettier C, Martinelli L, Regan M, Pavie A, Cabrol Ch *et al*. Cryopreserved small-diameter arterial allografts for arterial by-pass procedures: an experimental study. *J Cardiovasc Surg* 1995;**36**:79–85.

30 Dobrin PB, Mrkvicka R. Failure of elastin or collagen as possible critical connective tissue alterations underlying aneurysmal dilatation. *Cardiovasc Surg* 1994;**2**:484–488.

31 Patel MI, Hardman DT, Fisher CM, Appleberg M. Current views on the pathogenesis of abdominal aortic aneurysms. *J Am Coll Surg* 1995;**181**:371–382.

32 Mesh CL, Baxter BT, Pearce WH, Chisholm RL, McGee GS, Yao JS. Collagen and elastin gene expression in aortic aneurysms. *Surgery* 1992;**112**:256–261.

33 Zielinski T, Rynkun D, Broda G, Korevicki J. The diameter of the common carotid artery and intima-media thickness-independent or related variables? *Acta Angiol* 1999;**5**:97–103.

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